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基础研究

Tal1促进急性T淋巴白血病Jurkat细胞的增殖

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摘要:目的 探讨Tal1对T-ALL细胞增殖及其机制的影响。**方法** 用Tal1慢病毒感染T-ALL细胞株Jurkat,建立稳定的Tal1敲降细胞株(Jurkat-siTal1)和Tal1过表达细胞株(Jurkat-T1),及siRNA阴性对照细胞(Jurkat-mock1)和过表达阴性对照细胞(Jurkat-mock2)。CCK-8检测细胞生长能力;流式细胞术检测细胞周期;Real-time RT-PCR和Western blot检测周期蛋白依赖性激酶抑制因子2(CDKN2A)、周期蛋白依赖性激酶抑制因子1(CDKN2B)mRNA和蛋白表达。**结果** 成功建立Jurkat稳定转染细胞株。CCK8结果表明细胞Jurkat-T1与Jurkat-mock2相比,细胞生长更快,而Jurkat-siTal1与Jurkat-mock1相比,细胞生长明显减缓。流式细胞术检测细胞周期发现,Jurkat-siTal1与Jurkat-mock1相比G_0/G_1期增加,S期减少;而Jurkat-T1与Jurkat-mock2相比,G_0/G_1期减少,S期增加。Real-time RT-PCR和Western blot结果显示Tal1抑制Jurkat细胞内CDKN2A、CDKN2B的mRNA和蛋白表达。**结论** Tal1可以促进T淋巴白血病细胞Jurkat增殖;促进Jurkat细胞由G0/G1期向S期转换,其可能通过Tal1抑制G_0/G_1和S期负调控蛋白CDKN2A、CDKN2B的表达。
关键词: Tal1;Jurkat;细胞周期;CDKN2A;CDKN2B

Tal1 promotes proliferation of acute lymphoblastic leukemia Jurkat cells *in vitro*

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Abstract: Objective To investigate the role of Tal1 gene, which is aberrantly expressed in 40%-60% of patients with T lymphocytic leukemia (T-ALL), in the proliferation of T-ALL cells. **Methods** We established stable Jurkat-siTal1 and Jurkat-T1 cell lines by trasnfecting T-ALL Jurkat cells with lentiviral vectors to knock-down or overexpress Tal1. Jurkat cells transfected with negative control siRNAs for Tal1 knock-down (Jurkat-mock1) and over-expression(Jurkat-mock2) served as the control cells. The proliferation of the cells lines was assessed using CCK-8 assay, and the cell cycle distribution was determined by flow cytometry. The mRNA and protein expressions of cyclin-dependent kinase inhibitor 2 (CDKN2A) and cyclin-dependent kinase inhibitor 1 (CDKN2B) were measured by real-time RT-PCR and Western blotting, respectively. **Results** Jurkat-T1 cells showed more active proliferation *in vitro* than Jurkat-mock2 cells, while Jurkat-siTal1 cells showed slower growth than Jurkat-mock1 cells. In Jurkat-T1 cells, G_0/G_1 phase cells were decreased and S phase cells increased compared with Jurkat-mock2 cells, and Jurkat-siTal1 cells showed increased G_0/G_1 phase cells and decreased S phase cells compared with Jurkat-mock1 cells. Real-time RT-PCR and Western blotting showed that Tal1 inhibited the cellular expression of CDKN2A and CDKN2B at both mRNA and protein levels. **Conclusion** Tal1 promotes the growth and the transition from G_0/G_1 phase to S phase in T-ALL cells Jurkat by inhibiting the expressions of G_0/G_1 and S phase negative regulatory proteins CDKN2A and CDKN2B.
Key words: Tal1; T lymphocytic leukemia; cell cycle; CDKN2A; CDKN2B

白血病是儿童最常见的恶性肿瘤,严重威胁儿童健康。其中以急性淋巴细胞白血病最为常见。近年来,随着儿童白血病机制的研究和临床诊疗的发展,儿童急性B淋巴细胞白血病(B-ALL)患者的预后显著改善,其5年无病生存率达90%。但急性T淋巴细胞白血病(T-ALL)生存率仅为60%~75%,T-ALL发生发展的具体机制亟待研究[1]。

Tal1,急性淋巴细胞白血病蛋白同系物1,是Ⅱ类碱性/螺旋-环-螺旋(bHLHⅡ)转录因子家族的重要成员[2],是造血过程中重要的转录因子,其高表达占儿童T-ALL的40%~60%[3]。现有研究证实Tal1高表达的T-ALL恶性程度高,预后不佳,5年生存率约为40%[3]。有文献报道,Tal1可以促进T-ALL细胞的增殖[4-5],而相关机制仍不清楚。本课题组通过建立Tal1稳定转染的Jurkat细胞株,对其机制进行初步的研究,为后续的

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靶向治疗提供基础。

1 材料和方法

1.1 主要试剂及药物

RPMI 1640培养基和胎牛血清(Gibco);CCK-8试剂(凯基);anti-Tal1抗体(Cell Signaling)和anti-GAPDH(北京中衫金桥);anti-CDKN2A抗体(武汉三鹰);anti-CDKN2B(Santa Cruz);RIPA细胞裂解液(百泰克);Trizol RNA提取液(Takara);逆转录提取试剂盒(Takara);Tal1过表达、Mock和siTal1慢病毒载体为吉凯公司构建;SYBR Green Ⅱ 试剂(Takara);引物(华大基因)。

1.2 细胞培养

人急性T淋巴细胞性白血病细胞Jurkat细胞购自美国培养物集存库(ATCC)。细胞培养于含10%胎牛血清,青霉素100 U/mL和链霉素100 μg/mL的RPMI 1640培养液中,细胞培养于37 ℃ 5% CO_2条件中。

1.3 Tal1稳定转染细胞株的建立

在细胞培养对数生长期,准备96孔细胞板,3000细胞/孔;再根据Jukat的MOI值,加入6×10^4 U慢病毒载体,100 μL Optimum培养基,100 μL Enhancer,和37 ℃ 5% CO_2孵箱6 h。换10%胎牛血清,青霉素100 U/mL和链霉素100 μg/mL的RPMI 1640培养液;8 h后行镜下荧光观察GFP荧光表达的细胞比例,细胞扩大培养后,有限稀释法对荧光细胞的筛选,然后扩大培养即得稳定细胞株以备进一步验证Tal1的表达。

1.4 CCK8检测细胞增殖

取对数期细胞96孔板铺板,细胞3000/200 μL 1640完全培养基每孔,每24 h加入10 μL CCK-8试剂混匀37 ℃ 5% CO_2孵育3 h后,检测450 nm吸光度(D_{450} nm),连续监测5 d。

1.5 细胞周期检测

将待检测细胞去血清培养,同步化处理后,取2~5×10^5细胞用PBS洗2遍,弃上清,将细胞沉淀于70%乙醇混匀固定1 h,然后450 *g*离心,弃上清,PBS洗2次,每次5 min。避光加入碘化丙啶(PI)染液,暗室混匀静置30 min,使用流式细胞仪进行检测。

1.6 Western blot检测Tal1、CDKN2A、CDKN2B的表达

每50 μL细胞沉淀加入250 μL RIPA裂解液,冰上裂解30 min,每间隔5 min震荡混匀1次。4 ℃,12 000 r/min离心30 min,保留上清。用BCA法检测蛋白浓度,加入5×Loading Buffer混匀后85 ℃煮沸5 min,取20 μg蛋白SDS-PAGE电泳,PVDF膜转膜,5%脱脂奶粉室温封闭1 h,加入一抗(Tal1 1∶700、CDKN2A 1∶500、CDKN2B 1∶20、GAPDH 1∶20 000),4 ℃孵育过夜,次日加入二抗(1∶5000)室温孵育1 h,TBST充分洗涤后,用增强化学发光系统(ECL)检测膜上信号。

1.7 SYBR 荧光定量PCR法检测Tal1、CDKN2A、CDKN2B基因mRNA水平

(1)Trizol法提取细胞总RNA;(2)RNA逆转录为cDNA;(3)Real-time RT-PCR:反应体系:12.5 μL SYBR Ⅱ,10.5 μL ddH_20,1 μL cDNA,上下游引物(表1)各0.5 μL。反应条件:95 ℃预变性5 min;95 ℃ 20 s,58 ℃ 20 s,72 ℃ 30 s,循环35次,每个循环结束检测荧光。

表1 引物信息

Tab.1 Sequences of the primers for RT-PCR

Primer	Forward	Reversed
Tal1	TTCCCTATGTTCACCACCAA	AAGATACGCCGCACAACTTT
CDKN2A	AGGTCATGATGATGGGCAGC	CACCAGCGTGTCCAGGAAG
CDKN2B	GGGACTAGTGGAGAAGGTGC	CCCATCATCATGACCTGGATCG
GAPDH	CAGCGACACCCACTCCTCCACCTT	CATGAGGTCCACCACCCTGTTGCT

1.8 统计学处理

实验结果采用SPSS17.0统计软件进行分析,实验独立重复3次,所有数据统计显示方差齐,采用单因素分析,组间两两比较采用*t*检验,P<0.05认为差异有统计学意义。

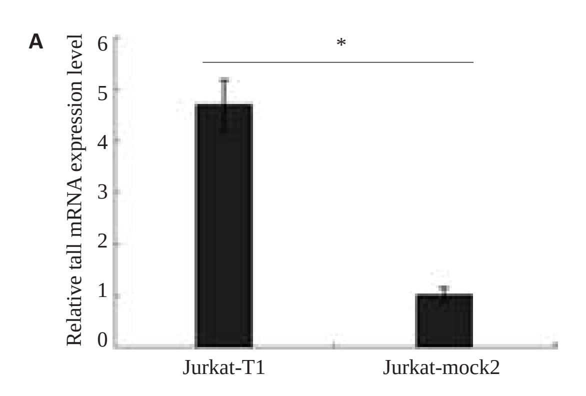
2 结果

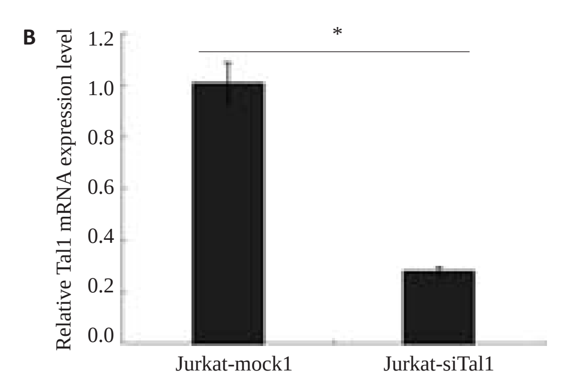
2.1 Jurkat稳转细胞株的建立及Tal1的表达鉴定

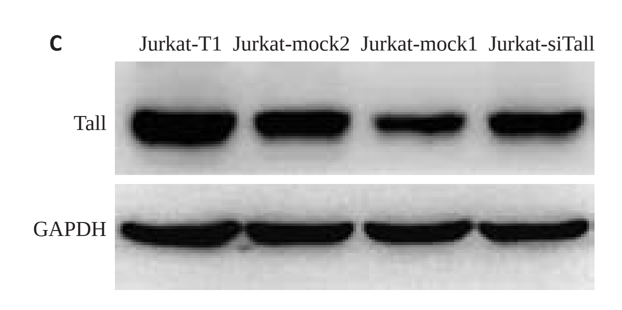
Real-time RT-PCR和Western Blot检测Tal1在Jurkat-siTal1、Jurkat-mock1、Jurkat-T1和Jurkat-mock2中mRNA和蛋白表达水平。结果显示Jurkat-siTal1中Tal1的mRNA水平明显下降,而Jurkat-T1细胞中的Tal1 mRNA水平明显升高(P<0.05)。Western Blot结果与Real-time RT-PCR结果一致(P<0.05,图1)。

2.2 Tal1促进Jurkat细胞生长

利用CCK-8实验连续监测5 d Jurkat-T1、Jurkat-mock2、Jurkat-siTal1和Jurkat-mock1组的A_{450}值,结果显示Jurkat-siTal1细胞中A_{450}值从第2~5天显著低于Jurkat-mock1细胞,而Jurkat-T1细胞的A_{450}值从第2~5天显著高于Jurkat-mock2细胞(P<0.05,图2)。







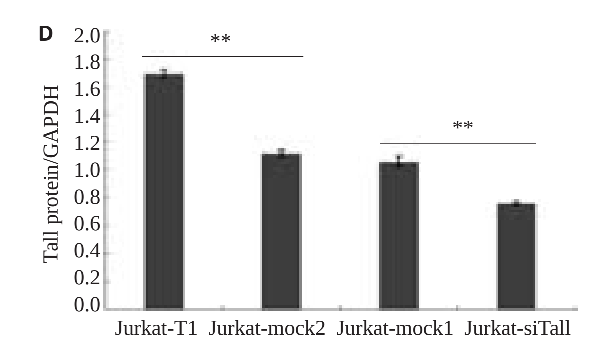


图1 Jurkat稳转细胞株的建立及Tal1的表达鉴定

Fig.1 Expression of Tal1 in Jurkat cells infected by lentivirus vectors. *A*, *B*: Real-time RT-PCR results for Tal1 mRNA expression standardized against GAPDH; *C*: Western blotting for Tal1 protein expression with GAPDH as the loading control. siTal1-control served as mock1 and T1-control as mock2; *D*: Statistical analysis of the protein levels in different Jurkat cells. **P*<0.05, ***P*≤0.001.

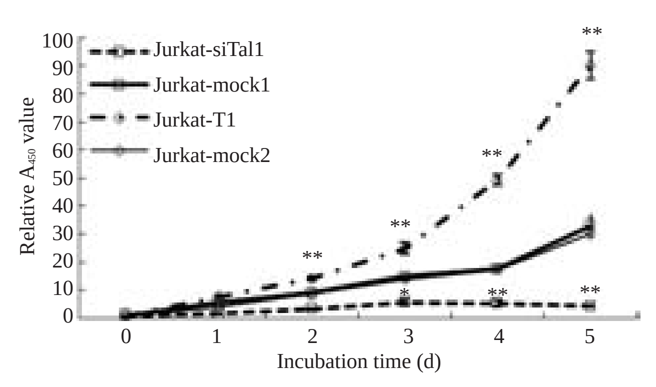
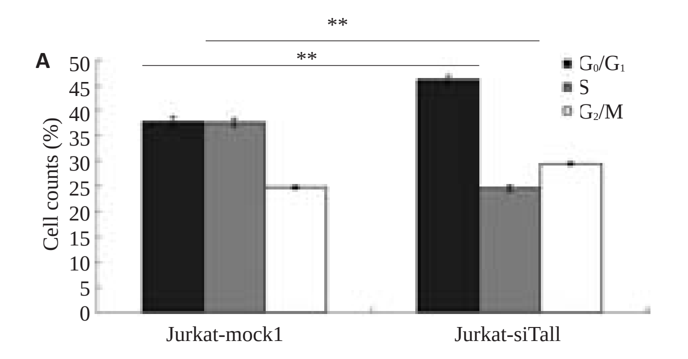


图2 Tal1促进Jurkat细胞增殖

Fig.2 Statistical graph of cell proliferation in different Jurkat cells with CCK8 assay. **P*<0.05 Jurkat-siTal1 *vs* Jurkat-mock1; ***P*≤0.001 Jurkat-T1 *vs* Jurkat-mock2.

2.3 Tal1促进Jurkat细胞G_0/G_1期向S期转换

使用流式细胞术PI染色检测不同Jurkat稳定转染细胞株的对数生长期24h时的细胞周期，结果显示细胞周期在Jurkat-siTal1与Jurkat-mock1相比时，G_0/G_1期显著增多，而S期显著减少，说明Jurkat-siTal1组G_0/G_1期向S期的转换明显受到阻滞，而Jurkat-T1与Jurkat-mock2细胞周期相比，G_0/G_1期显著减少，而S期显著增多，说明Jurkat-T1组G_0/G_1期向S期的转换明显增加（*P*<0.05，图3）。



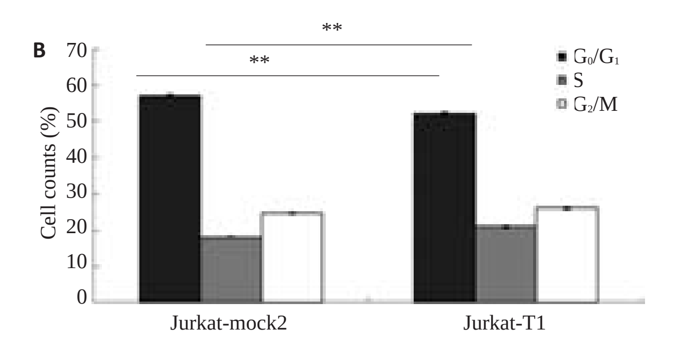


图3 Tal1促进Jurkat细胞G_0/G_1期向S期转换

Fig.3 Tal1 promotes cell cycle transition from G_0/G_1 phase to S phase in Jurkat cells. *A*, *B*: Statistical graph of cell cycle in different Jurkat cells. **P*<0.05. ***P*≤0.001.

2.4 在Jurkat细胞中Tal1抑制CDKN2A和CDKN2B基因的表达

通过Real-time RT-PCR检测发现,Jurkat-siTal1与Jurkat-mock1组相比,CDKN2A和CDKN2B的mRNA表达水平显著增高,而Jurkat细胞中Tal1过表达后CDKN2A和CDKN2B的mRNA表达水平均显著降低。通过Western blot检测发现Jurkat-siTal1组CDKN2A和CDKN2B的蛋白表达水平显著增高,Jurkat-T1组CDKN2A和CDKN2B的蛋白表达水平显著降低(P<0.05,图4)。

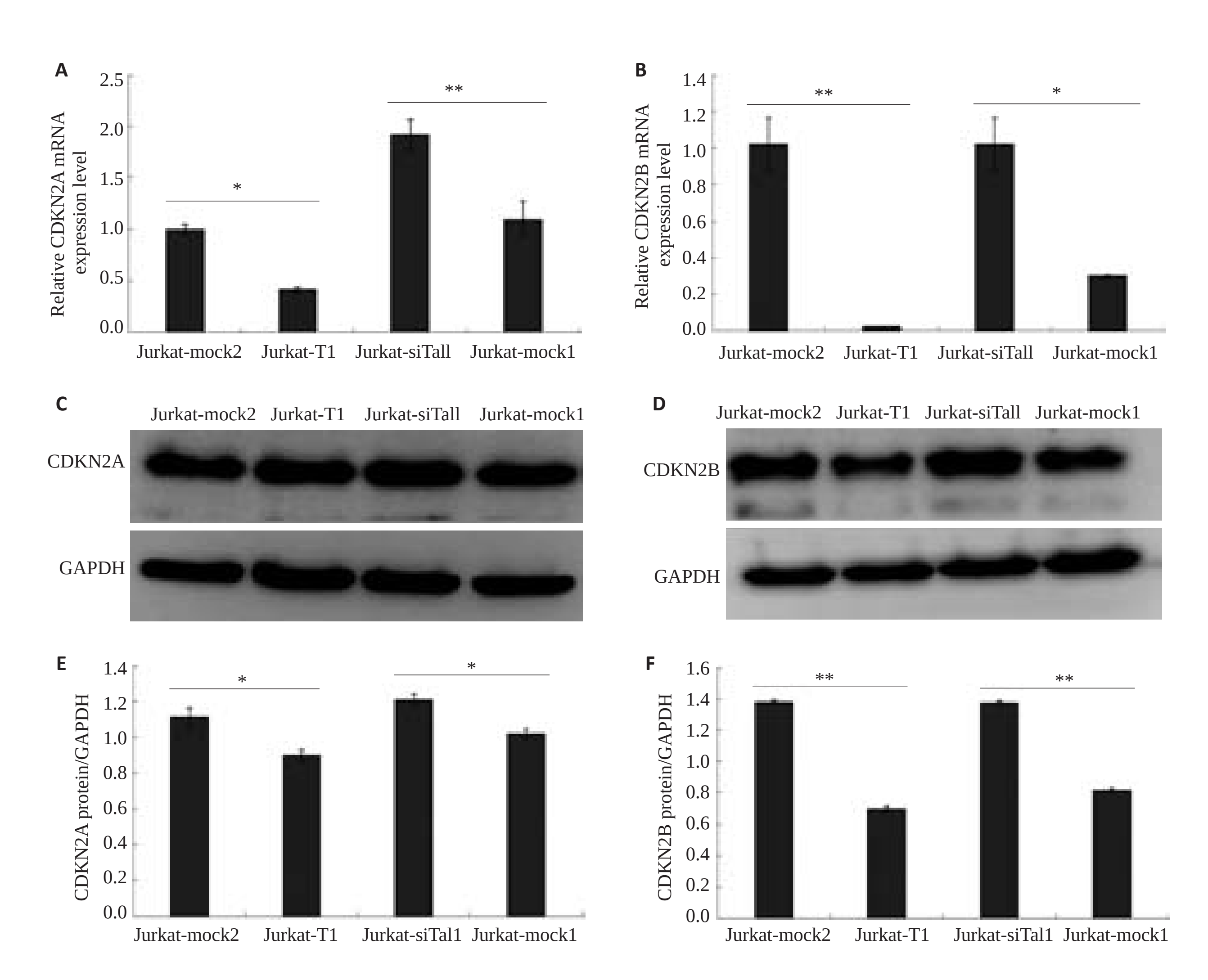


图4 Jurkat细胞中Tal1抑制CDKN2A和CDKN2B表达

Fig.4 Tal1 inhibits genes expression of CDKN2A and CDKN2B in Jurkat cells. ***A, B***: Real-time RT-PCR results for CDKN2A and CDKN2B mRNA expression; ***C, D***: Western blotting for CDKN2A and CDKN2B protein expression with GAPDH as control. siTAL1-control served as mock1 and T1-control as mock2; ***E, F***: Statistical graph of protein level in different Jurkat cells. *P<0.05. **P≤0.001.

3 讨论

急性淋巴细胞白血病是儿童最常见的恶性肿瘤,分为急性B淋巴细胞白血病(B-ALL)和急性T淋巴细胞白血病(T-ALL),T-ALL发病率比B-ALL低,仅占ALL的15%~20%,但其预后不良[6]。目前研究发现,在65%的T-ALL病人中发生了Tal1的异常表达,且这种异常表达经常导致染色体的易位和缺失[7]。Tal1是Ⅱ类bHLH家族的一种转录因子,它在造血早期具有重要的作用[8]。Tal1也是一种致癌基因,有文献报道Tal1的异常表达与T-ALL的不良预后有密切关系[9]。在我们的研究中Tal1表达促进T-ALL Jurkat细胞增殖与进展,也说明Tal1与T-ALL的发生发展有密切的关系。

文献报道,Tal1过表达对一些细胞周期有促进作用[10-13]。我们检测了Jurkat稳定转染细胞的细胞周期,发现Tal1在细胞分裂间期能够促进G_0/G_1期向S期转换。G1期在生长因子的刺激下,CyclinD表达,并能够与CDK4、CDK6结合形成复合物,从而促进G_1期向S期的转换,而细胞内具有一组CDK抑制因子,例如CDKN2A和CDKN2B,是CDK4特异性抑制物,能够与CyclinD竞争性结合CDK4,从而抑制细胞周期G_1向S

期转换[13-15]，在我们的研究中，利用PCR和Western blot技术检测不同Jurkat稳定转染细胞株中CDKN2A和CDKN2B的表达，发现在Tal1过表达的Jurkat细胞中CDKN2A和CDKN2B表达显著降低，而在Tal1敲低组中CDKN2A和CDKN2B的表达显著升高，这些研究表明Tal1可能通过抑制CDKN2A和CDKN2B表达，来促进Jurkat细胞的增殖，为后续的研究奠定基础。

在本研究中，Jurkat-siTal1与mock1细胞株相比，CDKN2A和CDKN2B mRNA和蛋白的表达升高，而Jurkat-T1与mock2细胞株相比，CDKN2A和CDKN2B的表达下降，但mRNA水平变化明显，蛋白水平变化不是很明显，这可能是由于两个原因引起的，首先，真核基因表达的转录和翻译发生的时间存在时空间隔，mRNA达到峰值时蛋白量还在增加中；其次，在转录后又会有转录后加工、转录产物的降解、翻译、翻译后加工及修饰等，因此，转录水平和翻译水平并不完全一致。有研究者对124个T-ALL病例进行分析时发现，有64%肿瘤因为发生了CDKN2A基因重排，因此在DNA水平上其表达是缺失的；在超过60%肿瘤中CDKN2A基因在mRNA水平上也不表达[16]，这些发现说明了CDKN2A的缺失在T-ALL中具有重要作用。当然，Tal1也可能通过不同的信号通路来影响细胞的增殖。现有文献报道，Tal1在细胞内环境的作用下可以与不同的复合DNA亚基结合，引起白血病的发生。 Tal1与急性髓细胞白血病1蛋白(RUNX1)和ETS1(P54)能够发生相互作用，这些转录因子是Tal1调节T细胞分化时所要结合一些基因时所必需的，Tal1通过抑制T细胞分化从而引起T细胞肿瘤的形成[17]。在人类肿瘤和老鼠模型中，Tal1高表达与胸腺细胞$CD4^+CD8^+$阶段分化阻滞相关，而Jurkat细胞是$CD4^+CD8^+$T-ALL细胞，而且TAL1异常表达，在多种T-ALL分子研究中被应用[2, 5]，所以在我们的研究中应用了Jurkat细胞作为研究对象。Jurkat细胞属于WT型Tal1高表达的细胞株，为了更确切的说明Tal1对T-ALL细胞增殖的影响，下一步我们还需要选择一株Tal1缺失型细胞株HPB-ALL做进一步研究。

综上所述，本文发现Tal1可以通过抑制CDKN2A和CDKN2B表达来促进Jurkat细胞的增殖。但Tal1促进Jurkat细胞增殖的机制非常复杂，其他相关机制需要进一步研究。

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